DETAILED ACTION

Applicants' response of 6/15/2009 has been received and entered into the application file. Claims 3 and 27 have been amended; claims 19-22 and 24-26 have been cancelled. Claims 2-18, 23 and 27 remain pending in the instant application, of which claims 2-4, 8-14, 16, 17 and 27 have been considered on the merits. Claims 5-7, 15, 18 and 23 remain withdrawn from consideration pursuant to 37 CFR 1.142(b) as being directed to non-elected species of the invention, no generic claim having been found yet allowable. Election was made with traverse in the reply of 8/23/2008.

Response to Arguments/Amendments

Applicants' remarks received 6/15/2009 have been fully considered in combination with the amendments. Remarks pertaining to maintained rejections are each addressed below. Rejections/objections not repeated herein have been withdrawn.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 3, 12 and 13 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 3 remains indefinite, as it is not clear that claim 3 properly correlates to parent claim 27. Claim 3 defines the step of cultivating the non-human blastocyst to comprise preparing the cells of the internal cell mass (ICM) of the blastocyst in a culture dish or preparing a soluble matrix fraction from the cells (of the ICM), and further defines the step of supplying the differentiable human donor cells to

comprise placing the donor cells [into] the culture dish or soluble matrix fraction. As stated in the previous rejection, the language of claim 3 appears to require separation and culture of cells from the ICM of the blastocyst, not the entire blastocyst; this does not correlate with the method of the parent claim, which requires supplication of the human donor cells to the blastocyst. The blastocyst must remain intact in order for cells to be supplied to the blastocyst. Furthermore, claim 3 also now defines supplying the donor cells to the blastocyst as comprising supplying the donor cells to the culture dish (in which the separated ICM cells have been cultured) or to the soluble matrix fraction; again, neither the cultured, separated ICM cells nor the soluble matrix fraction are the blastocyst, thus claim 3 appears to be redefining the claimed method. Clarification is required.

In claim 12 it is unclear what properties or characteristics the cells of ICM actually demonstrate to result in their reduced survivability. Claim 12, which depends from claim 9 and ultimately claim 27, requires the internal cell mass of the blastocyst to comprise cells whose genome contains vectors that cause a lethal sensitivity to appropriate cultivation conditions in comparison to the wild type, wherein the survivability of the cells of the ICM of the blastocyst to be reduced in a way that is tailored to the varying degrees of differentiation of the donor cells and is chronologically well-ordered. While it is understood that the genome of the cells of the ICM may comprise a vector that causes a lethal sensitivity to specific culture conditions, it is unclear how (or if) this lethal sensitivity is tailored to the varying degrees of differentiation of the donor cells? The correlation between the reduced survivability of the cells of the ICM and the degree of differentiation of the donor cells is unclear, thus it is unclear how one is to be tailored to meet the other. It does not appear that the lethal sensitivity has anything to do with the degree of differentiation of the donor cells, but rather depends solely on the culture conditions. Furthermore, it is not clear what is meant by "chronologically well-ordered"? What is chronologically well-ordered? the reduced survivability of the cells of the ICM? the donor cells? Clarification is required.

In claim 13 it is unclear how the high contact affinity between the donor cells and blastocyst is measured, and furthermore, what is considered sufficiently 'relatively high' in order to support selection. "Relatively high contact affinity" is a relative term which renders the claim indefinite. The term "relatively high" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. Clarification is required.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Applicants have traversed the rejection of record based on Geiger et al, in view of Tsukamoto et al and Eggan et al under 35 USC 103(a) on the following grounds: (a & b) Geiger et al does not teach using human donor cells, and specifically not human bone marrow cells, but rather murine bone marrow donor cells, and claim 27, as amended requires the donor cells to be human donor cells. (c) Geiger et al teaches murine donor HSCs from bone marrow, not umbilical cord blood; Applicants traverse the holding that HSCs from bone marrow are equivalent to HSCs from bone marrow on the grounds that this holding is unsupported. (d) Geiger et al does not teach the cells of the ICM to have reduced survivability; Applicants traverse the holding that selection of blastocysts with reduced or restricted survivability would have been *prima facie* obvious in order to improve the take-rate of the donor cells because Geiger et al identify other factors which are critical to the take-rate of the donor cells, specifically the age of the

blastocyst. Applicants further assert Geiger et al had the benefit of the teachings of Eggan, regarding use of blastocysts with restricted survivability, and thus the failure of Geiger et al to refer to such method is evidence of nonobviousness. (e) Applicants assert substitution of blastocysts having reduced survivability in the method of Geiger et al would have been non-obvious because such would result in expansion of the injected stem cells over the host cells, which Applicants assert Geiger et al hypothesizes would be undesirable. (f) With respect to Eggan, Applicants assert the Examiner has mischaracterized the teachings of Eggan, specifically that Eggan does not teach donor cells injected into a tetraploid blastocyst are ultimately without competition and thus the donor cells will give rise to all cells of the developing embryo, But rather that Eggan teaches a chimeric embryo will result. (g) Finally, Applicants assert that Geiger emphasizes that the developmental stage of the hematopoietic microenvironment controls the developmental fate of the transplanted progenitor cells, thus a reduction or restriction of survivability of the host blastocyst would have not been suggested or expected to provide successful results.

It is initially noted that Applicants' argument at points (a) and (b), that Geiger et al does not disclose donor cells from human bone marrow, but rather donor cells from murine bone marrow, and thus cannot properly be applied against the current claims, which require human donor cells, are persuasive. Geiger et al was inadvertently mis-cited. The rejection of record based on Geiger et al is thus withdrawn.

The remaining points of argument are moot in view of the new grounds of rejection.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner

to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 2, 4, 8-11, 14, 16, 17 and 27 are rejected under 35 U.S.C. 103(a) as being unpatentable over Harder et al (Blood, 2002), in view of Wang et al (Mechanisms of Development, 1997).

Harder et al disclose a method for producing human hematopoietic cell lines by injecting human cord blood-derived hematopoietic stem cells (HSCs) into murine blastocysts.

Specifically, Harder et al report injecting human hematopoietic stem cells derived from human umbilical cord blood into gestational day 3.5 (E3.5) murine blastocysts; the blastocysts were then reimplanted into murine foster mothers. At day E11.5 single cell suspensions were obtained from the chimeric embryos and cultured *in vitro* in the presence of human growth factors to produce expanded cell colonies. (See Harder et al, Pg. 719, "Study Design"). 17(alpha)mod PCR revealed at least some (8/93) of the colonies were of human origin (See Harder et al, Pg. 721, "Following injection, human CB CD34+ CD38- cells give rise to hematopoietic progenitors in fetal liver and embryonic blood" and Fig. 1C).

The method of Harder et al is comparable to the method of the instant invention as follows:

The murine blastocysts read on non-human blastocysts. The blastocysts were harvested at 3.5 days gestation, which is considered to read on the claimed step of 'cultivating a non-human blastocyst under conditions that enable further development of the blastocyst to occur in stages in which newly formed cell lines having a high degree of differentiation are produced' (claim 27).

The human hematopoietic stem cells (HSCs) read on differentiable human donor cells which are naturally occurring stem cells from umbilical cord blood (claims 2 & 4). The HSCs were isolated on the basis of CD34+/CD38- expression. The expression of the CD34 cell marker is considered to read on "a genetic marker that ensures cells having a lower degree of differentiation are isolated and supplied to the

blastocyst" (claim 14), as CD34 is a hematopoietic stem cell marker and hematopoietic stem cells are less differentiated than mature hematopoietic cells.

Injection of the HSCs into the blastocysts reads on the step of 'supplying the differentiable human donor cells to the blastocyst' (claims 27 and 17).

Preparation of single cell suspensions, and further expansion of the single cell suspensions into colonies reads on the step of 'isolating the cell lines' (claim 27).

The method of Harder et al differs from the instant invention in that the cells of the internal cell mass (ICM) of the murine blastocysts do not have a reduced or restricted survivability compared to corresponding wild-type blastocyst ICM cells.

The method of Harder et al has the potential to generate large quantities of human hematopoietic cells *in vitro* (i.e. the cell lines identified as being of human origin separated and expanded from the embryonic tissue). Furthermore, because the human hematopoietic cells are derived from the transplanted donor cells, the resulting hematopoietic cells have the same genotype as the donor cells; thus the method of Harder et al has the potential to generate large quantities of human hematopoietic cells having specific genotypes, including specific genetic mutations and/or HLA types. Such a method would have been recognized as desirable for research purposes, for example, generation of HLA-matched blood cells for transplantation back into the original donor.

However, in the method of Harder et al a relatively low percentage of the expanded cell colonies were reported to be of human origin (8/93) (See Harder et al, Pg. 721 "Following injection, human CB CD34+ CD38- cells give rise to hematopoietic progenitors in fetal liver and embryonic blood" and Fig.

1C). Harder et al report the host blastocyst did not provide a selective advantage for the injected HSCs (See Harder et al, Pg. 721, second column, second full paragraph).

In order to increase the proportion of cells which are of donor (human) origin, which would have been desired as discussed above, it would have been *prima facie* obvious to one of ordinary skill in the art to utilize tetraploid blastocyst complementation assays. Tetraploid blastocyst complementation was a recognized technique in the art whereby a chimera comprising donor cells are formed through injection of donor stem cells into tetraploid blastocysts. Because the tetraploid cells of the blastocyst have only limited potential in post implantation development, the donor cells are favored in development of the chimera (See Wang et al, Pg. 137, "Introduction"). Means of generating tetraploid blastocysts for chimera production were well known in the art (See, e.g. Wang et al, Pg. 138, "2.2 Viable ES mice generated by tetraploid embryo aggregation and blastocyst injection," first paragraph). Therefore, in using tetraploid murine blastocysts in the method of Harder et al, one would have had a reasonable expectation of successfully increasing the number of donor (human) cells obtainable from the chimeric embryos, as the host cells would have reduced survivability, and the donor cells would have been favored. Tetraploid blastocysts read on what Applicants call blastocysts wherein the cells of the ICM have restricted survivability in comparison to corresponding wild-type cells (wild-type cells being diploid) (claims 27 and 8).

It is further submitted that other methods of genetically altering the survivability of the ICM of the blastocyst, either by introduction of a vector which codes for a gene which reduces survivability of the endogenous ICM cells, through use of antibodies against the native ICM cells, or addition of an antibiotic or additive to which the donor cells are resistant, would also have been well within the purview of one of ordinary skill. Methods of genetic engineering of cells, including blastocysts were well known in the art, and thus the artisan of ordinary skill would have been able to successfully produce a blastocyst and/or

donor cell with the desired resistance, or susceptibility to various culture conditions, antibiotics, or antigens, as required to impart the desired reduced survivability to the cells of the ICM of the blastocyst, for the reasons discussed above. (claims 9-11)

Finally, it is noted the murine blastocysts differ from those required by instant claim 16, as they are not pig blastocysts. However, it is submitted that the animal source of the blastocyst would have been a matter of experimental design choice. Both porcine and murine cells were available at the time the invention was made, and thus selection of either animal source would have been within the purview of the artisan of ordinary skill. (claim 16).

Therefore the invention as a whole would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ALLISON M. FORD whose telephone number is (571)272-2936. The examiner can normally be reached on 8:00-6 M-Th.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Michael Wityshyn can be reached on 571-272-0926. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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/Allison M. Ford/
Examiner, Art Unit 1651